Analysis of HIV Seropositive Thalassemic Children for Antibodies Specific to *Aspergillus fumigatus* by Luminescent Immunoassay

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The applicability of luminescent immunoassay (LIA) in serodiagnosis of fungal infections in multitransfused (MT) thalassemic children seropositive for human immunodeficiency virus (HIV) was investigated. Thirty-one sera samples from HIV infected pediatric patients with thalassemia receiving repeated blood transfusions were analysed for the presence of antibodies specific to *Aspergillus fumigatus* by LIA. The LIA was standardized using well defined antigens of *A. fumigatus*. Ten out of 31 (32.2%) of the MT-HIV positive patients were found to have anti-*Aspergillus* antibodies in their sera by LIA. The ELISA could detect *A. fumigatus* specific antibodies in

25.8% (8 out of 31) of the patients. Thus, 20% more number of patients turned to be positive for aspergillosis by LIA as compared to ELISA. The difference was found to be statistically significant ($p < 0.005$). Of the MT-HIV negative patients only 1 out of 33 (3%) showed *A. fumigatus* specific antibodies by LIA and ELISA both. In age and sex matched control group ($n = 25$) none of the patients was found to be positive for antibodies to *A. fumigatus*. LIA was found to have better discriminatory value indicating, thereby, its utility in diagnosis of aspergillosis in compromised patients. J. Clin. Lab. Anal. 11:343–345, 1997. © 1997 Wiley-Liss, Inc.

Key words: *Aspergillus fumigatus*; human immunodeficiency virus; thalassemia; luminescent immunoassay; multitransfused

INTRODUCTION

Aspergillus fumigatus infection is increasingly recognized as an emerging serious public health hazard. The systemic infection due to *A. fumigatus* in immunocompromised patients may become fatal if not managed properly (1). *A. fumigatus* infection has been considered to be a cofactor for enhancement of disease progression in HIV infected individuals (2). We recently observed that the specific antibodies to *A. fumigatus* were present in 25% of multitransfused patients seropositive for human immunodeficiency virus (HIV) as compared to only 2% among HIV seronegative multitransfused children (3). The multitransfused children of thalassemia major positive for HIV were considered to be at added risk of complications due to infection with *A. fumigatus* (3). The impairment of antifungal activity of neutrophils without evidence of neutropenia (4–6), may be an important cause for *Aspergillus* induced complications in HIV infected individuals. Therefore, identification of presence of *A. fumigatus* in such conditions using ultrasensitive methods will be helpful in better management of patients. The detection of specific IgG and IgE antibodies in the sera of patients were reported to be useful in serodiagnosis of aspergillosis (7–9). However, less sensitive assay systems developed using un-

defined antigens have limited value in serodiagnosis of aspergillosis in patients with impaired immune system (10). In the present communication we describe the usefulness of an ultrasensitive luminescent immunoassay (LIA) in serodiagnosis of *A. fumigatus* infection in multitransfused thalassemic children with HIV infection.

MATERIAL AND METHODS

Sera Samples

Sera samples were obtained from following three groups of pediatric patients.

Group I. Sera from 31 multitransfused (MT) children seropositive for HIV. The competitive enzyme immunoassay (Welcozyme recombinant, Wellcome Diag. UK) and confirmatory western blotting (Du Pont, USA) were performed as described in our previous study (11) to ascertain the seropositivity for HIV.

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TABLE 1. Incidence of Specific IgG to *A. fumigatus* in Multitransfused HIV Infected Children

Group	Total No. of samples	Positive by ELISA		Positive by LIA	
		No.	Percent	No.	Percent
MT-HIV seropositive	31	8	25.8	10	32.2
MT-HIV seronegative	33	1	3.03	1	3.03
Control	25	0	0	0	0

No. = Number

Group II. Sample included 33 sera from MT children who were seronegative for HIV.

Group III. It was a control group in which 25 age and sex matched nontransfused healthy children were the source of sera samples.

Enzyme-linked Immunosorbent Assay (ELISA)

Basic technique of Voller (12) as modified by Banani et al. (13) was used to carry out indirect ELISA for detection of IgG and IgE type of antibodies specific to *A. fumigatus*. Briefly the ELISA was performed in polystyrene flat bottom microtitre plates (NUNC, Denmark) using 1 µg/well of *A. fumigatus* antigen for coating. Anti-human IgG coupled to horse radish peroxidase was used to probe the *A. fumigatus* specific antibodies. The plate was read at 490 nm on an ELISA Reader (Immunoreader NJ-2000 Nunc, GMBH). The ELISA absorbance ratio (normal to patient) 1:3 and above for specific IgG was considered as positive evidence for aspergillosis (14–16).

Luminescent Immunoassay (LIA)

LIA was performed in ECL immunoassay microtitre plates (Amersham International U.K. RPN 189) as described else-

where (14). After adding 100 µl/well of freshly prepared ECL-immunoassay signal reagent (Amersham International, U.K. RPN 190), the photo emission from the wells was recorded at a dwell time of one second using Amerlite Luminescence Analyser.

RESULTS AND DISCUSSION

In the present investigation a total number of 64 sera samples (31 seropositive for HIV and 33 seronegative for HIV) from multitransfused children were analysed for the presence of *A. fumigatus* infection by using ELISA and LIA. The incidence of anti-*A. fumigatus* antibodies has been shown in Table 1. It was observed that 25.8% (8 out of 31) of the HIV positive MT children were positive for *Aspergillus* infection by ELISA where as LIA could detect *A. fumigatus* specific antibodies in 32.2% (10 out of 31) of the patients. All the samples (sample numbers 4-P, 7-P, 11-P, 12-P, 16-P, 19-P, 23-P, and 29-P) that were positive for aspergillosis by ELISA were positive by LIA also. However, two samples (Nos. 5-P and 22-P) that were not detected by ELISA, turned to be positive for *A. fumigatus* infection when analysed by LIA (Table 2). The percent positivity for aspergillosis in MT-HIV seronegative patients was found to be only 3% by LIA as well as ELISA both (Table 2). Follow up observations revealed that one of the patients (No. 16-P) who was positive for *A. fumigatus* infection by LIA progressed from HIV to AIDS related complications and died.

In the current study it was observed that 20% more number of sera samples from MT-HIV seropositive patients were positive for *A. fumigatus* infection by LIA as compared to ELISA. The detection of *Aspergillus* specific antibodies in more number of patients by LIA was possible due to the fact that LIA has been reported to have higher sensitivity and better discriminatory value (16). The screening of MT-HIV positive children using LIA would, thus, help in identifying

TABLE 2. Positivity of MT-HIV Infected Pediatric Patients for *A. Fumigatus* Antibodies by ELISA and LIA

Group	Sample Number	ELISA			LIA		
		OD 490nm	Ratio C:pt.	Interpretation	Emission	Ratio C:Pt.	Interpretation
MT-HIV +ve	4-P	0.33	3.31	+ve	209.02	5.22	+ve
	5-P	0.25	2.51	-ve	199.80	4.99	+ve
	7-P	0.47	4.66	+ve	493.49	12.33	+ve
	11-P	0.53	5.53	+ve	288.38	7.20	+ve
	12-P	0.92	9.12	+ve	658.52	16.46	+ve
	16-P	0.77	7.61	+ve	369.93	9.24	+ve
	19-P	0.33	3.35	+ve	325.75	8.14	+ve
	22-P	0.21	2.14	-ve	247.65	6.19	+ve
	23-P	0.39	3.93	+ve	586.21	14.65	+ve
	29-P	0.37	3.23	+ve	340.94	8.52	+ve
MT-HIV -ve	8-N	0.36	3.57	+ve	253.85	6.34	+ve
Control pool		0.10	—	-ve	40.00	—	-ve

C - Control sample

Pt. - Patient

opportunistic fungal infection in immunocompromised host at early stage.

It has been demonstrated (3) that exposure to *A. fumigatus* may act as a cofactor in the disease progression from HIV to AIDS. In view of this even detection of two more *A. fumigatus* patient is worth emphasising. It is difficult to ascertain whether these patients had invasive aspergillosis or not as these *A. fumigatus* +ve children had normal chest X-ray and had no evidence of neutropenia. However, it is very likely they have had subclinical infection. To our surprise, among MT HIV +ve children, one who was -ve for *A. fumigatus* antibodies by ELISA was found to be +ve for *A. fumigatus* by LIA and this patient developed AIDS with fatal outcome. These findings suggest that the blood used for transfusion, irrespective of seropositivity for HIV, should also be screened for *A. fumigatus* infection. The presence of antibodies to *A. fumigatus* by LIA could be demonstrated in 32.2% of MT children seropositive for HIV as compared to 25.8% by ELISA. The LIA, therefore, may be a test of choice for screening of immunocompromised patients for presence of antimycotic antibodies.

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